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Possible transmission of viruses from contaminated human feces and sewage: Implications for SARS-CoV-2

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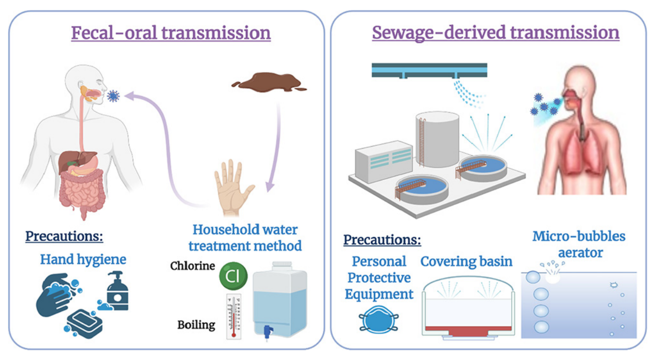
HIGHLIGHTS

- Hand hygiene protects from the transmission of COVID-19 via fecal-Oral route.
- Personal protection tools are essential for workers at wastewater treatment plant.
- Virus occurrence in wastewater enable to estimate the spread of the infected cases.
- Aerosols might infect workers if infectious SARS-CoV-2 arrived to WWTP.

GRAPHICAL ABSTRACT



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ABSTRACT

Humanity has experienced outbreaks by viruses such as severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) in 2003, Eastern respiratory syndrome coronavirus (MERS-CoV) in 2012, Ebola virus in 2014 and nowadays SARS-CoV-2. While clinicians seek for a vaccine to reduce the epidemic outbreak, environmental engineers need to understand consequence of virus entity in sewage given the reported persistency of viruses in human feces and sewage environments for more than days. Herein, we discuss about concerns associated with virus occurrence in human feces and sewage, with attention to the possible SARS-CoV-2 transmission routes, based on the review of recent studies on SARS-CoV-2 as well as the previous pandemic events. Given the reported environmental stability of coronavirus, the feces- and sewage-derived transmission routes may be of importance to prevent unprecedented spread of coronavirus disease 2019 (COVID-19) particularly in developing countries. However, so far, limited number of studies detected infectious SARS-CoV-2 even in human feces, whereas a number of virus RNA copies were identified in both feces and sewage specimens. Therefore, uncertainty remains in the possibility of this transmission pathway, and further investigation is warranted in future studies, for example, by increasing the number of specimens, examining the effectiveness of methods for viral viability test, considering the patient medical history, and so forth.

1. Introduction

Nowadays, there is an increasing global fear of viral pandemics in the hyperconnected world. In 1918, Spanish influenza H1N1 pandemic caused deaths number exceeding 50 million people (Taubenberger

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and Morens, 2008). Since then, we have experienced viral pandemics such as SARS, MERS, and Ebola virus disease with 774, 456 and 10,353 fatalities, respectively (ECDC, 2015; Ge et al., 2013; WHO, 2020a). Some respiratory viruses such as SARS-CoV-1 and MERS-CoV affiliated to *Coronaviridae* family are known as zoonotic virus which can cause infection in both animal (e.g., bats) and human as virus host (Woolhouse and Gaunt, 2007). Emergence of mutation further causes high risk of transmission from person to person in the absence of immunity against novel strain (Wigginton et al., 2015). In addition, the global travel expanding, growing demand for food (especially animal market), and illegal trade of endangered species, may facilitate virus spreading and evolution (Naidoo and Fisher, 2020). These facts combined with the strong contagious nature as well as the environmental persistency can be, at least partially, related to the unprecedented pandemic of the novel SARS-CoV-2 (posing the coronavirus disease 2019, namely, COVID-19), which has infected about 19.5 million people and caused 722,285 deaths at minimum (according to World Health Organization [WHO] situation report accessed on 10 August 2020) (WHO, 2020a).

The SARS-CoV-2 infection has also been spreading in developing countries including African countries. For example, increasing number of infection cases are confirmed in South Africa (553,188 cases), Egypt (95,314), Nigeria (46,140), Ghana (40,533), Algeria (34,693) and Morocco (32,007) (accessed on 10 August 2020). The number of infection cases are also growing in the other African countries (WHO, 2020a). Consequently, particular concerns should be given to developing countries for mitigating the spread of SARS-CoV-2 especially in low-income and densely populated area that suffer from limited healthcare facilities, as well as limited access to safe water and sufficient hygiene (Brauer et al., 2020). Nearly 60% of Sub-Saharan Africa population subsist in slum conditions with lack of basic sanitation such as sewer collection networks. Nairobi and Uganda, for example, use mainly latrines, small-bore sewers and natural wetlands for the sewage treatment. The coverage of centralized wastewater treatment technologies (e.g., activated sludge, trickling filters, stabilizing lagoons and oxidation ditch) are limited in Africa (Wang et al., 2014). According to the recent report of United Nation (UN), Egypt and Tunisia sustain 51 to 75% of safely treated wastewater, while Morocco has 26 to 50% of achievement with the rest of African countries having either less than 25% or insufficient data (WHO, 2018).

While SARS-CoV-2 is transmitted mainly from person to person via respiratory droplet and contagious routes (thus community behavior is a key in the pandemic trajectory) (Naddeo and Liu, 2020; La Rosa et al., 2020a), this respiratory virus is also confirmed to be excreted in human waste (Chen et al., 2020a). Thus, fecal-oral contamination route may be one of concerns in developing countries holding insufficient sanitation system (Lodder and de Roda Husman, 2020). Further, even in the countries with safe sanitation systems such as wastewater treatment plants (WWTPs), sewage-associated virus transmission routes are still reported to exist for some viruses (e.g., via inhalation of virus-loaded sewage aerosols) (Brinkman et al., 2017; Drossinos and Stilianakis, 2020), despite the fact that current technologies used in sewer system can be effective in safe transport of sewage and removal of some pathogens (Qiao et al., 2018; Ye et al., 2018). Accordingly, we herein discuss concerns on the occurrence of viruses in human feces and sewage with attention given to the possible SARS-CoV-2 transmission via fecal-oral route and sewage-derived aerosol routes, which is of particular concern in the developing countries. We also discuss the challenges in future study and summarize precautions for the possible fecal- and sewage-derived transmission.

2. Fecal-oral transmission

To obtain viable and infectious virus particles in feces, virus must survive after experiencing variety of chemically harsh environments. In the gastrointestinal tract, virus structure will be in contact with the stomach and bile acids. Furthermore, virus needs to keep infectious after released to the environment to infect a new host (Bushman et al., 2019). The presence of coronaviruses in the gastrointestinal tract was previously confirmed. Their resistance against digestive enzymes as well as lower pH environment is most likely due to the strong interaction between mucins and S protein (Holmes, 2001; van Doremalen et al., 2013). Indeed, 76 and 25% of patients infected by SARS-CoV-1 and MERS-CoV, respectively, experienced the gastrointestinal symptoms (i.e., diarrhea) during their illness period (Assiri et al., 2013; Kwan et al., 2005), and infectious SARS-CoV-1 was isolated from the intestinal tissue (Lee et al., 2003). Similarly, MERS virus was reported to reproduce in the human intestinal enteroids and enterically transmitted to transgenic mice (hDPP4) (which is similar to human tissues; e.g., kidney and lung) (Zhou et al., 2017).

SARS-CoV-2 has been reported to recognize angiotensin-converting enzyme 2 (ACE2) as its viral receptor in human gastrointestinal tract (Arslan et al., 2020). Xiao et al. (2020a, 2020b) discovered that ACE2 protein is plentifully expressed in the glandular cells of gastric, duodenal, and rectal epithelia, though it is rarely seen in esophageal mucosa. Additionally, Zang et al. (2020) explained that co-expression of Transmembrane Serine Protease (TMPRSS2 and TMPRSS4) with ACE2 facilitated the entry of SARS-CoV-2 into host cells in the human small intestine. Such enteric infection can be accompanied by symptoms of nausea or vomiting (10.2% of total infected cases) and diarrhea (12.5%) (Cheung et al., 2020; Guan et al., 2020; Hindson, 2020). The excrement from virus-infected human has virus loads ranging from 10^5 to 10^{13} copies per gram of feces in case for viruses listed in Table 1 (with daily human sheds approximately 100 g feces/person) (Fechner et al., 2013; Timm et al., 2013). In case of SARS-CoV-2, the PCR-positive feces' specimens in France had the virus load of $10^{6.8}$–$10^{8.1}$ copies/g (note that the positives were 2 out of 5 patients investigated) (Lescure et al., 2020). Urine samples were free of SARS-CoV-2 in the same study, though the study in China suggested possible occurrence of the virus in urine in case of severe infection (Zhang et al., 2020b). In any cases, however, the feces samples frequently hold the viral RNA (e.g., 10 out of 12 feces samples with average concentration of 5623 copies/mL in case of the study in China) for a long period (e.g., extending 22 days of disease, and interestingly, the virus remained in fecal even 26 days after the patient recovery) (Zhang et al., 2020b).

Kingsbury et al. (2020) hypothesized that nasal passage mucus ingestion is the reason behind the occurrence of high RNA copies of SARS-CoV-2 in the gastrointestinal tract, though this is unlikely the case. For example, it is reported that those who became negative with nasopharyngeal RT-PCR test persistently showed positive in the rectal swabs test (Chen et al., 2020a; Hindson, 2020; Zhang et al., 2020b). Further, Wu et al. (2020) demonstrated that viral load shed from the digestive tract was usually higher than that for the respiratory tract. Therefore, the continuous positive detection of viral RNA from feces suggests that the infectious viruses are secreted from the virus infected gastrointestinal cells (Xiao et al., 2020b).

To date, some studies reported that infectious SARS-CoV-2 was detected from the PCR-positive stool specimens mainly using the method of Vero cells, as listed in Table 2 (Jeong et al., 2020; Wang et al., 2020; Xiao et al., 2020b, 2020a; Zhang et al., 2020a). Among these studies, the study by Wang et al. (2020) detected infectious virus in stool specimens of patients even with no symptoms of diarrhea. In contrast, infectious virus was undetectable from the treatments of urine and stool specimens using the Vero cells in the study by Jeong et al. (2020), though viral RNA loads were higher than 20 copies/ml. Interestingly, ferrets intranasally inoculated with the same stool and urine specimens witnessed the increased body temperature and rhinorrhea, suggesting the occurrence of infectious virus undetectable by the Vero cells.

Zang et al. (2020) simulated the reactions taken place in gastrointestinal tract using gastric fluid [FaSSGF at pH 1.6], small intestinal fluid [FaSSIF-V2 at pH 6.5] and colonic fluid [FaSSCoF at pH 7.8] (purchased from Biorelevant.com Ltd) to mimic the human gastrointestinal conditions. The authors found that the low pH of gastric fluids significantly reduced the infectious ability of virus after 10 min of exposure. In addition,

Table 1
Previous studies on virus occurrence in human feces, sewage and sewage-associated aerosols.

Virus	Structure and diameter of the virus	Disease and viral load at human feces	Virus concentration in wastewater	Virus concentration in aerosols released from wastewater
SARS-CoV-2	Enveloped (+) ssRNA virus with diameter ~ 50–200 nm (Chen et al., 2020b).	The virus causes respiratory and enteric symptoms (Yeo et al., 2020). It was found in fecal samples with different concentration range from $10^{5.6}$ to $10^{8.1}$ copies/g (Lescure et al., 2020; Zhang et al., 2020b).	The survival of SARS-COV-2 in wastewater was confirmed by some studies (Lodder and de Roda Husman, 2020; Wurtzer et al., 2020). A French study found that virus concentration in wastewater was directly proportional to number of fatal cases in range from 10^5 to $10^{6.5}$ copies/L (Wurtzer et al., 2020). The lifetime is not determined.	Recent study found the virus in toilets' aerosols with concentration of 19 copies/m^3. High viral loaded aerosols was associated with the aerodynamic diameter in the range 0.25–0.5 μm (Liu et al., 2020).
SARS-CoV-1	Enveloped (+) ssRNA virus with diameter ~ 60–220 nm (Gundy et al., 2009)	The virus causes respiratory and enteric symptoms and its viral load at human feces was found to be around 10^7 copies/g (Hung et al., 2009).	Average concentration of SARS-CoV-1 was determined to be 144 plaque-forming units (PFU)/mL in wastewater (Wang et al., 2005). The life time of SARS-CoV-1 in wastewater was 2–3 days with $\log_{10}$ reduction ranging from 2.0 to 3.4 (Gundy et al., 2009)	SARS-CoV-1 was found in wastewater aerosols during Hong Kong outbreak in 2003. The airborne aerosols were generated from leaked pipe, resulting in the infection of 319 cases (Naddeo and Liu, 2020; Wigginton et al., 2015).
Norovirus	Nonenveloped (+) ssRNA virus with diameter ~ 23–40 nm (Hasegawa et al., 2017)	The virus causes gastroenteritis (with symptom of vomiting and diarrhea). Viral load at human fecal sample is 10^9 copies/g (Lee et al., 2007).	The reported virus concentration at WWTPs is ≤10^9 copies/L (Wigginton et al., 2015).	Among tested, 9 out of 16 wastewater aerosol samples were positive with highest virus concentration of 3.2×10^2 copies/m^3. Samples were collected from grit chamber and aeration tank of activated sludge with sampling place 80 cm higher than the wastewater surface (Matsubara and Katayama, 2019) Aerosol samples were collected from two sites near the rack of grit removal chamber and aeration tank, and 3 out of 123 samples were found to be positive (though concentrations were under detection limit). Sampling place was 1.5 m above the basin's surface (Masclaux et al., 2014).
Adenovirus	Nonenveloped dsDNA virus with diameter ~ 80–90 nm (Delmdahl, 2006)	Respiratory and gastrointestinal diseases are caused by infection with this virus. Infected human stool has adenovirus load in the range from 10^5 to 10^{13} copies/g (Elmahdy et al., 2019).	The reported virus concentration in wastewater is ≤10^8 copies/L (Wigginton et al., 2015).	Aerosol samples were collected from 1.5 m above of grit removal chamber and aeration tank water surface. Positive samples were 123 out of 123 and maximum virus concentration was 2.27×10^6 copies/m^3 (Masclaux et al., 2014)
Rotavirus	Nonenveloped dsRNA virus with diameter ~ 75 nm (Yates, 2014)	Rotavirus causes gastroenteritis with diarrhea and was found at high concentrations up to 10^{10} copies in gram of feces (Ruggeri et al., 2015).	Virus concentration in wastewater ranges ≤10^7 copies/L (Wigginton et al., 2015).	Air samples were collected from four WWTPs, where 6 out of 10 samples were positive, and maximum and minimum virus concentrations were 2.2×10^5 and 1.7×10^4 copies/m^3, respectively (Brisebois et al., 2018)
Ebolavirus	Enveloped (−) ssRNA virus with diameter ~ 80 nm and its length is around 1 μm (Vingolo et al., 2015).	Ebola disease causes severe diarrhea and virus human feces was found at around 10^7 copies/mL (Lin and Marr, 2017).	Ebolavirus can survive for 1 day in wastewater and ~ 94% remains in the mobile liquid phase (Lin and Marr, 2017).	Concentration of Ebola model viruses (MS2 and Phi) in aerosols was investigated, indicating that virus was not loaded at aerosols released from the toilet, while virus was detected in the aerosols released from aeration chamber at concentrations of 20 and 0.1 plaque-forming units (PFU)/L for MS2 and Phi, respectively (Lin and Marr, 2017).

although synthetic small intestinal and colonic fluids have neutral pH of 6.5 and 7.8, the infective titers of SARS-CoV-2 significantly decreased (only small number of infectious SARS-CoV-2 was detected after 24 h) due most likely to the bile and digestive enzymes in small intestine, as well as the dehydration and exposure to multiple bacterial byproducts synthesized in the colon. Despite the findings by Zang et al. (2020), some studies detected infectious SARS-CoV-2 in stool samples (Table 2). The reason behind can be that Zang et al. (2020) utilized the fixed pH for gastrointestinal fluids, while the fluid pH can be variable from person to person, for example, due to the atrophic gastritis or utilization of proton pump inhibitor medications (ELetters-Science Immunology, 2020).

Chan et al. (2020) tested virus stability in different environmental conditions by releasing the infectious virus to external environment via fecal shedding. The results indicated that SARS-CoV-2 remained viable for 7 days in solution at room temperature (25 °C), while the virus lost its infectious ability within 1 day at warmer temperature of (37 °C). Further, the authors demonstrated that SARS-CoV-2 was unable to survive at pH 2 and 3, whereas the virus remained viable for 1 day in conditions of pH 4 and 11. Further, in pH range from 5 to 9, SARS-CoV-2 lost its infectivity by 2.9 and 5.3 logs after 6 days. The authors also found that the virus remained viable for 1–2 days in watery stool specimen at room temperature (25 °C) with loss of infectivity by 5 logs.

Table 2
Studies that detected infectious SARS-CoV-2 in human excreta.

Specimen types	Genetically positive result relative to all samples	Occurrence of infectious viruses	Methods	References
Stool	44 of 153 (29%)	Not quantified	Detection and quantification of RNA were performed using reverse transcription-polymerase chain reaction (RT-PCR) and real-time RT-PCR (RT-PCR). Virus isolation in Vero cells and subsequent electron microscopy observation was performed to detect live virus	(Wang et al., 2020)
Stool	6 of 20 (30%)	2 of 6 (33%) Note: Patients did not have diarrhea.		
Urine	Not detected	Not detected		
Stool	39 of 73(53%) Note: Positive stool results spanned from 1 to 12 days after commencement of illness. In addition, 17 (23%) patients remained to have positive results in stool after showing negative results in respiratory samples.	Infectious SARS-CoV-2 detected (not quantified)	RNA was detected via RT-PCR tools. Viral nucleocapsid staining was performed using laser scanning confocal microscopy	(Xiao et al., 2020b)
Stool	12 of 28 (42.8%) Note: Viral load was higher in feces than in respiratory specimens collected at multiple time sampling (i.e., 17–28 days after symptom onset)	2 of 3 (66.67%)	Detection and quantification of SARS-CoV-2 RNA were performed via RT-PCR tools. Vero cells were used for virus isolation.	(Xiao et al., 2020a)
Stool	1 of 1 (100%)	1 of 1 (100%)	Virus was isolated using Vero cells, and then detected using electron microscopy	(Zhang et al., 2020a)
Stool	5 of 5 (100%) Note: Virus loads were $1.17 \pm 0.32 \log_{10}$ copies/ml	Occurrence of SARS-CoV-2 was not detected in urine and stool samples via Vero cells though the specimens had high viral loads. However, intranasally inoculated ferrets with urine and stool specimens witnessed increase in body temperature and rhinorrhea, showing the occurrence of infectious virus.	Detection and quantification of SARS-CoV-2 RNA were performed via RT-PCR devices. Vero cells were used for virus isolation. Cells were monitored daily for 4 days to examine the cytopathic effects. Infection of ferrets with clinical specimens' method was used to assess the occurrence of infectious virus.	(Jeong et al., 2020)
Urine	5 of 5 (100%) Note: Virus loads were 1.08 ± 0.16–$2.09 \pm 0.85 \log_{10}$ copies/ml			

In summary, the possibility of fecal-oral infection remains in debate. Thus, further research is needed to examine possible occurrence of fecal-oral transmission. Particularly, the patient medical history should be recorded, since the credibility of the result can be affected by patient health condition and associated prescription (e.g., drugs to facilitate digestion, reduce the acidity of stomach, or to treat the colon). Moreover, studies on oral transmission of coronaviruses (i.e., foodborne or waterborne ingestion) are still insufficient and further research is necessary (Hoseinzadeh et al., 2020; Olaimat et al., 2020). Therefore, the occurrence of fecal-oral transmission route should be warranted in further detailed studies for SARS-CoV-2, even though there are no reports on the presence of fecal-oral transmission.

Another concern is the inhalation or aspiration of fecal particles, as well as its direct contact with skin and eyes (Hoseinzadeh et al., 2020). During the SARS-CoV-1 outbreak in Hong Kong, 2003, the leakage in household sewage pipe was likely associated with 319 infection cases due to the spread of aerosolized fecal particles (Naddeo and Liu, 2020; Wigginton et al., 2015). Dense interconnected sewage pipes via tees, ferrules and other fitting devices may have high opportunities to be busted and leaked, causing the spread of contaminated sewage. Thus, some precautionary measures to prevent clogging of plumbing system such as use of U-bend pipes at bathrooms and kitchen has been highly recommended in such cases. Controlled sealing of all pipe connection should be also periodically checked (Gormley et al., 2020). Due to the insufficient compliance with aforementioned precautionary measures, the COVID-19 group infection occurred in a high-rise building located in Guangzhou, China, where nine infected patients were identified in three families living in three vertically aligned flats connected by drainage pipes in the master bathrooms. The PCR-positive environmental samples generally suggest the vertical spread of virus-laden aerosols (Conticini et al., 2020). Further, Fears et al. (2020) demonstrated that SARS-CoV-2 remained infectious for 16 h in the aerosol based on the laboratory experiment.

In addition to the sewage pipes, other household sanitation facilities such as pressure-assisted toilets are considered as possible sources of COVID-19 infection. A study at two different Wuhan hospitals investigated the airborne SARS-CoV-2, showing high viral load (19 copies/m^3) in aerosols at the toilets zone and the continual use of disinfection was forced to inactivate the virus in aerosols (Liu et al., 2020). The same study recommended to flush the toilet with the lid down where available and minimize the usage of public bathrooms until this pandemic pass away (Liu et al., 2020; WHO, 2020). Li et al. (2020) also simulated the trajectories of aerosol particles during flushing the toilet, and found that 40%–60% of particles reach above the toilet seat (107 cm from the ground), potentially leading to the large-scale dispersion of virus in atmosphere. More recent study by Feng et al. (2020) investigated virus occurrence in aerosols from healthcare facilities found that SARS-CoV-2 RNA was positive for 2 (out of 6) samples with concentrations of 4 and 2 copies/cm^2 after defecation and toilet flushing, respectively. In addition, the virus RNA was detected for 1 (out of 6) sample with concentration of 2 copies/cm^2 at floor of drainage system.

Least develop countries (LDCs) that suffer from the lack of basic sanitation infrastructure can be more susceptible to the spread of the anticipated fecal-oral or fecal-inhalation transmission routes especially for the countries where COVID-19 has been already or started spreading (Ober, 2020). The risk of exposure to the contaminated wastewater aerosols can be increased in shared sanitation practice for crowded informal settlements in developing countries (Gwenzi, 2020). LDCs consist of 47 countries, and 33 of them are in the middle Africa. In most of these African countries, less than 25% of people can access to safely managed sanitation, and there are totally two billion people worldwide lack with basic sanitation (WHO, 2018). Regardless of the debates on occurrence of fecal-oral transmission, caution needs to be taken to reduce the risk of virus outbreak via this route particular for developing countries with low healthcare facilities and poor sanitation as well as lack of awareness about potential risk (Crocker et al., 2016a; Miesler et al., 2020; Tilley et al., 2014). Table S1 summaries some of general lessons obtained from previous studies in developing countries to mitigate the health risk from fecal contamination. Briefly, human hands (particularly on children's hand) can be highlighted as a major vector in the fecal-oral transmission route for most developing countries owing to some specific habits such as open defecation (Clasen et al., 2012). Personal hygiene is also commonly considered as the major protective shield against virus infection via fecal transmission route (Crocker et al., 2016b; Rah et al., 2015). Consequently, governments of developing countries should provide adequate amount of soap and increase the awareness of keeping the hand clean towards citizen. Other important vectors include contamination of water storage devices with sewage as consequence of improper sanitation practice. For example, household water treatments (HWT) such as boiling and chlorination are recommended in rural areas, though the selected methods should fit to the user adherence to grantee its effective use (Lantagne and Clasen, 2012; McGuinness et al., 2020).

Further, WHO currently supports LDCs' governments with providing adequate number of instruments for SARS-CoV-2 detection as well as increasing the capacity of health-care professionals to improve their surveillance ability (Payne, 2020). More effort, however, should be made from the governments in terms of spreading health awareness and emphasizing on the personal hygiene between the communities to suppress the probability of COVID-19 spread via multiple routes.

3. Sewage-derived transmission

The occurrence of infectious SARS-CoV-2 in human excreta (i.e., feces and urine in severe cases) indicates the potential of virus survival in our sewage systems (Collivignarelli et al., 2020). The viability of enveloped viruses in wastewater was previously investigated. For example, Ye et al. (2016) found that enveloped viruses (murine hepatitis virus and *Pseudomonas* phage Φ6 as models) retained their infectious ability for a day in wastewater, where 26% of the two enveloped viruses were adsorbed to the solid fraction. In case of Ebola virus (another enveloped virus), 90% inactivation was observed after 2 days' residence in domestic wastewater. This significant reduction is likely due to the adsorption of viral particles to wastewater particles (Bibby et al., 2015). On the other hand, the period when 90% inactivation was achieved for phage Φ6 (enveloped virus model) varied from 24 min to 117 days depending on the temperature and composition of aqueous media employed (Aquino De Carvalho et al., 2017). Regarding the first SARS pandemic, SARS-CoV-1 RNA was detected in the sewage of hospitals treating the infected patients. The inactivation of SARS-CoV-1 was assessed under different conditions. For example, SARS-CoV-1 was found to retain its viability at hospital sewage for 2 weeks at 4 °C, and 2 days at 20 °C (Wang et al., 2005). Further, lifetime of SARS-CoV-1 (~3-log reduction) was determined to be 2–3 days in sewage and up to 10 days in tap water at 23 °C (Gundy et al., 2009). Regarding SARS-CoV-2 (which shares 82% genetic similarity with SARS-CoV-1 (Arslan et al., 2020)), this virus is reported to have the environmental stability comparable to SARS-CoV-1 (Taylor et al., 2020).

Apart from its viability, occurrence of SARS-CoV-2 RNA in wastewater has been reported in a number of recent studies. In Amsterdam, for example, SARS-CoV-2 RNA was detected in wastewater by using RT-PCR in 4 days after the emergence of first COVID-19 case (Lodder and de Roda Husman, 2020). Similarly, the quantitative measurement of SARS-CoV-2 RNA at three major WWTPs in Paris witnessed the increase of viral gene abundance, which corresponded to the reported number of fatal cases in upstream cities (Wurtzer et al., 2020). Therefore, increasing interest and attention have been given to the wastewater surveillance for epidemic warning (Ahmed et al., 2020; Hata and Honda, 2020; Kitajima et al., 2020; Noise, 2020; La Rosa et al., 2020b).

Other important concerns include virus infection via inhalation of sewage aerosols that may occur if inhaled aerosols retain the sufficient concentration of infectious virus (LeChevallier et al., 2020). There is a causal relation between aerosol sizes and infection symptoms, in which aerosols with aerodynamic diameters ranging from 5 μm to 10 μm were found to be able to adhere the nose and oral cavity causing disease (i.e., rhinitis), in case that aerosols carry viruses capable of infecting the entourage (Tellier et al., 2019). The aerosol with diameter less than 5 μm can reach the alveoli and smaller particles less 3.3 μm have been identified as respirable fraction with higher infectious risk to the lower respiratory tract (Han et al., 2020; Tellier et al., 2019).

Mechanisms of aerosol generation at WWTPs were well investigated in previous studies, though little studies were addressed in context of the virus occurrence (note that virus occurrence in sewage-derived aerosol is described below). In the aeration process, a large assortment of aerosols is potentially formed. For example, when a bubble with diameter ~ 600 μm is injected from the bottom of the reactors, it ruptures at the water surface to approximately five small droplets (diameter ~ 60 μm) forming aerosols that may travel up to ~8 cm above the water surface (Piqueras et al., 2016). However, the aerosols generation can be affected by operational parameters such as aeration rate and diffuser type, as well as, weather conditions (i.e., temperature and humidity) (Noh et al., 2019). Further, Bauer et al. (2002) indicated emission of airborne aerosols is reactor type dependent, where the aeration reactor of activated sludge showed higher aerosols emission compared to the biological fixed oxygen reactor (e.g., fixed biofilm reactor type with open-air water surface). In addition to the aeration reactor for activated sludge treatment, aerosol release was also observed from coarse screen, aerated grit chamber, primary settling tank and reactors with mixing devices such as sludge dewatering devices, (Han et al., 2018; Yang et al., 2019).

Regarding the mitigation of aerosols formation at WWTPs, Bauer et al. (2002) advised to reduce the surface area of the reactor where possible, which potentially prevents the spread of infection via inhalation, skin-contact, and ingestion. Additionally, micro-bubbles aerators are capable of reducing the wastewater aerosol generation compared to macro-bubbles type (Temesgen et al., 2017). The micro-type also has the benefits of oxygen content enrichment and higher oxygen transfer rate in the liquid phase (Terasaka et al., 2011). There are various type of micro-bubbles generators (i.e., spiral liquid flow, venturi, ejector and pressurized dissolution types) that can sustain oxygen bubbles with diameter in the range of 10–50 μm (Parmar and Majumder, 2013).

While different environmental stability of enveloped virus (e.g., coronaviruses and Ebola virus) from non-enveloped virus (e.g., norovirus, adenovirus, and rotavirus) should be carefully considered, non-enveloped viruses are often detected in sewage aerosols (Table 1). For example, adenovirus was earlier detected in airborne aerosols collected from 31 WWTPs in Switzerland especially from screening site and aeration tank (Masclaux et al., 2014). In many of countries (particularly developing countries), WWTPs generally consist of basic primary and secondary treatment stages, where most of such reactors (or tanks) are not covered: thus higher emission of sewage-derived aerosol is expected (Awad et al., 2019). A previous study in Japanese WWTPs (equipped with grit chamber, closed aeration [activated sludge] reactor and settling tank) indicated that the virus-contaminated aerosols (i.e., noroviruses and adenoviruses) were highly released from grit chamber, whereas the closed aeration reactor substantially reduced virus-loaded aerosol formation showing the important role of closed system in mitigating contaminated aerosol formation (Matsubara and Katayama, 2019). On the other hand, the study by Haas et al. (2017) on Ebola virus as enveloped virus suggests the higher risk of Ebola virus infection via inhalation of sewage aerosols holding the virus.

Practitioners in WWTPs were previously infected with non-enveloped virus via inhalation of wastewater aerosols. For example, at the secondary treatment zone in Canadian WWTP, three workers were infected by hepatitis A virus owing to high abundance of contaminated aerosols (De Serres and Laliberté, 1997). Moreover, 37% of the workers in Danish WWTP suffered from gastrointestinal symptoms, which was likely caused by the inhalation of sewage aerosols loaded with noroviruses. Indeed, 1.4×10^3 copies of noroviruses were collected from the aerosols using dust filter, which was orders of magnitudes higher than the infectious dose (e.g., 18 viral copies) (Uhrbrand et al., 2011).

Until now there are no reports showing infected cases by enveloped viruses, though Casanova et al. (2009) highlighted the potential of infection by coronavirus via viral-loaded aerosol (given that its infectious ability remains for a relatively long period). Recent study indicated that the viability of SARS-CoV-2 (i.e., the cytopathic effect) in Italian WWTP was not observed even in the presence of high amount of RNA copies (Rimoldi et al., 2020). The authors also noted that the viability of SARS-CoV-2 transported to WWTPs depends mainly on residential time of feces and other environmental conditions in the sewer network (Rimoldi et al., 2020). This study was, however, argued by Michael-Kordatou et al. (2020), since the authors utilized a small volume (2 mL) of wastewater samples without positive controls. Therefore, further investigation would be required to understand the possibility of infectious SARS-CoV-2 occurrence in wastewater. Enyoh et al. (2020) stated the concern that boosted circulation of the SARS-CoV-2 in human society may increase the probability of the infection via sewage-derived transmission practically viral aerosols inhalation as indirect exposure route to the virus.

Another interesting aspect may be relation of relative humidity (RH) with survivability of phage Φ6 in aerosol, with previous study showing the negligible effect of RH on the virus viability at RH of <33% and 100% (Lin and Marr, 2020). In contrast, when RH was at intermediate level (e.g., at RH of 75%) the virus viability was reduced (Lin and Marr, 2020), showing the possible importance of ambient humidity control in virus transmission in WWTPs. While the mechanism of inactivation at intermediate RH is still unclear, one of the plausible reasons may be due to the shrinkage and loss of activity caused by osmotic pressure (possible accumulation of salts in droplets) (Choi et al., 2015). The other suggested that the defects occurred at capsid structure may be the reason behind humidity-driven inactivation (Colas de la Noue et al., 2014).

Finally, some of general precautions are listed in Table S2 for protection of practitioners from the infection via inhalation of contaminated aerosols during the wastewater treatment. Briefly, the essential protection tool such as personal protective equipment (PPE) (i.e., working [outer] wear, masks, face shield, boots gloves, etc.) should be supplied (WHO, 2020). Other procedures could be taken, where possible, such as covering the grit removal chamber and aeration tank to prevent the release of aerosols into the air. Further, micro-bubbles generators can be more suitable (compared to macro-bubbles type) to mitigate the aerosol emission (by reducing the amount of bubbles that reach the water surface) (Temesgen et al., 2017).

CRediT authorship contribution statement

Mohamed Elsamadony: Conceptualization, Visualization, Writing - original draft. **Manabu Fujii:** Conceptualization, Supervision, Writing - review & editing. **Takayuki Miura:** Conceptualization, Writing - review & editing. **Toru Watanabe:** Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142575.

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